Highly enantioselective DNA-based catalysis

Roelfes, Gerard; Boersma, Arnold J.; Feringa, Bernard

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Supplementary Information
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"Highly Enantioselective DNA-based Catalysis"

by

Gerard Roelfes, Arnold J. Boersma and Ben L. Feringa

Figure S1. Induced CD spectra from $[Cu(L_{4\text{-}7})(NO_3)_2]$ (150 μM) combined with salmon testes DNA (0.8 mg/ml) in Mops buffer (20 mM, pH 6.5). a) only st-DNA; b) $[Cu(bipy)(NO_3)_2]$ / st-DNA; c) $[Cu(dppz)(NO_3)_2]$ / st-DNA; d) $[Cu(dpq)(NO_3)_2]$ / st-DNA; e) $[Cu(phen)(NO_3)_2]$ / st-DNA.

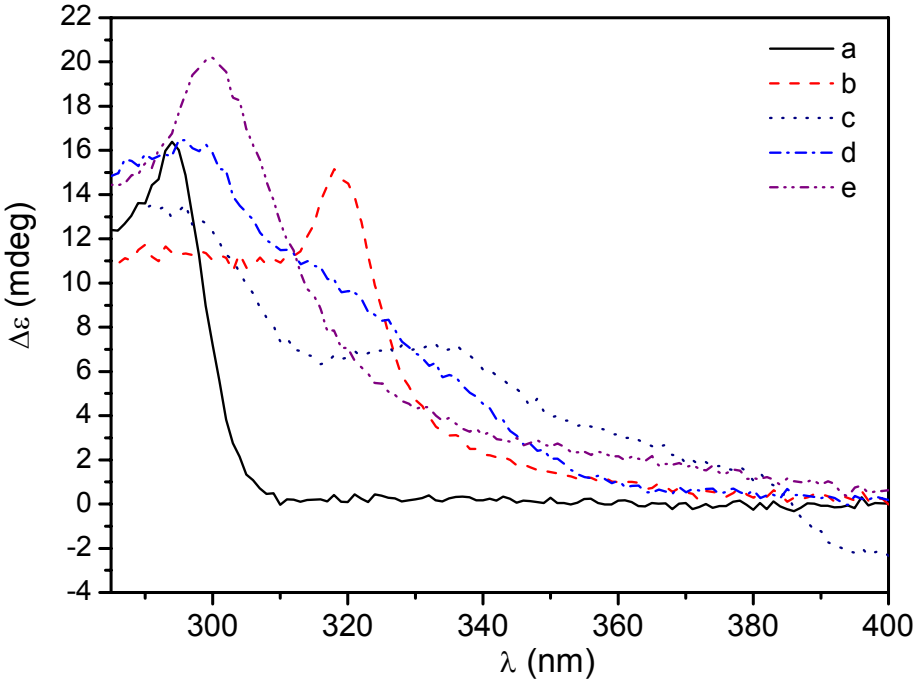

Table S1: Effect of variation of the concentration of $[Cu(bipy)(NO_3)_2]$, st-DNA and **2a** on the results of the catalyzed reaction.

Entry	Concentration $[Cu(bipy)(NO_3)_2]$ (mM)	Conc. DNA (mg/ml)	dienophile (mM)	Conversion (%)	Endo:Exo	E.e. Endo
1	0.05	1.3	1	>80	97:3	89
2	0.10	1.3	1	>80	98:2	91
3	0.15	1.3	1	>80	98:2	91
4	0.30	1.3	1	>80	98:2	89
5	0.45	1.3	1	>80	98:2	85
6	0.60	1.3	1	>80	98:2	77
7	0.15	0.65	1	68	98:2	89
8	0.30	0.65	1	>80	98:2	84
9	0.30	1.3	0.3	Quant.	97:3	90
10	0.30	1.3	1.5	>80	98:2	89
11	0.30	1.3	3	>80	97:3	87
12	0.30	1.3	6	65	97:3	86

Table S2

	catalyst	T	endo:exo	e.e. endo (%)
1[a]	$Cu(NO_3)_2$ / DNA	RT	95:5	10
2[b]	DNA	5 °C	n.d.[c]	<5 %
3[d]	$[Cu(dppz)(NO_3)_2]$ / DNA	5 °C	94:6	-

Table S2, Selection of relevant control experiments, performed under standard conditions. a) conversion 50-60 %. b) conversion < 5%. c) cannot be determined due to the low conversion. d)conversion 52 %.

Experimental and Synthetic Procedures

General remarks
Salmon testes and calf thymus DNA were obtained from Sigma.

Physical methods.

Equilibrium binding constants to salmon testes DNA were determined by UV/Vis titration, following the procedure of Meehan.[1] After dissolution of salmon testes DNA (2 mg/ml), the stock solution was dialyzed extensively against Mops buffer (20 mM pH 6.5) prior to use. The concentration in base pairs was determined spectrophometrically, using ε_{260} = 12800 M^{-1} cm^{-1}.[2] The absorbance ratio of $\lambda_{260}/\lambda_{280}$ was 1.8-1.9, indicating the DNA was sufficiently free of protein. The K_b was determined by titration of DNA to a solution of copper complex in buffered solution. Concentrations of copper complexes generally were 30 μM, or 15 μM in case of $Cu(dppz)(NO_3)_2$ and $Cu(dpq)(NO_3)_2$. Under conditions where the ratio of bound complex : DNA base pairs approaches zero, the K_b can determined using :

$$\frac{D}{\Delta\varepsilon_{ap}} = \frac{1}{\Delta\varepsilon} D + \frac{1}{\Delta\varepsilon K_b}$$

where $\Delta\varepsilon_{ap} = |\varepsilon_a - \varepsilon_f|$, $\Delta\varepsilon = |\varepsilon_b - \varepsilon_f|$, ε_a, ε_f and ε_b are the apparent, free and bound extinction coefficients for the complex, respectively, and D is the DNA concentration in basepairs. In a plot of $D/\Delta\varepsilon_{ap}$ vs. D, K_b is given by the ratio of the slope to the y intercept. A representative plot, for $[Cu(bipy)(NO_3)_2]$, is shown below.

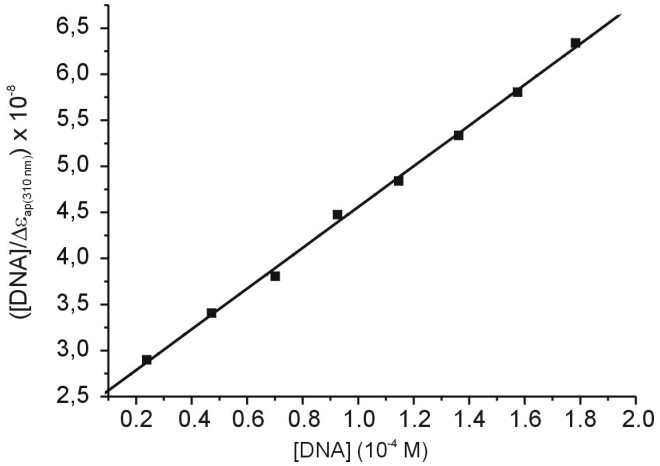

Figure S2. Representative plot of $[DNA]/\Delta\varepsilon_{ap}$ vs. [DNA] for $[Cu(bipy)(NO_3)_2]$ (30 μM). The solid line represents the least squares linear fit of the data.

Catalytic Diels-Alder reactions, representative procedure.

A buffered solution (20 mM Mops, pH 6.5) of DNA bound catalyst (1.3 mg/ml salmon testes DNA and 0.3 mM [Cu(bipy)(NO_3)$_2$]) was prepared by mixing a solution of salmon testes DNA (10 ml of a 2 mg/ml solution in 30 mM Mops, prepared 24 h in advance) with an aqueous solution of catalyst (5 ml of a 0.9 mM solution, prepared by adding a solution of [Cu(bipy)(NO_3)$_2$] in a minimal amount of dmso to 5 ml H_2O). An aliquot of a stock solution of dienophile **2a** in CH_3CN (30 μL of a 0.5 M soln., final conc. 1 mM) was added and the mixture was cooled to 5 °C. The reaction was started by addition of cyclopentadiene (21 μL, final conc. 15 mM) and mixed by continuous inversion for 3 days, followed by extraction of the product with diethyl ether. After H-NMR analysis the e.e. was determined by chiral hplc (Daicel chiralcel-ODH column). Selected products were purified by column chromatography and analyzed on a Daicel chiralcel-ODH column or Daicel chiralpak-AD column to confirm the results obtained from analysis of the crude product.
HPLC conditions:
3a: Daicel chiralcel-ODH, heptane/iPrOH 98:2, 0.5 ml/min. Retention times: 11.4, 12.3 (exo isomer), 13.7 and 16.7 mins (endo isomer); Daicel chiralpak-AD, heptane/iPrOH 99:1, 1 ml/min. Retention times: 9.7, 10.6 (exo isomer), 12.5 and 14.6 mins (endo isomer).
3b: Daicel chiralpak-AD, heptane/iPrOH 98:2, 1 ml/min. Retention times: 13.5, 15.2 (exo isomer), 17.4 and 21.1 mins (endo isomer).
3c: Daicel chiralcel-ODH, heptane/iPrOH 99.75:0.25, 0.5 ml/min. Retention times: 13.9, 14.7 (exo isomer), 20.4 and 25.3 mins (endo isomer).

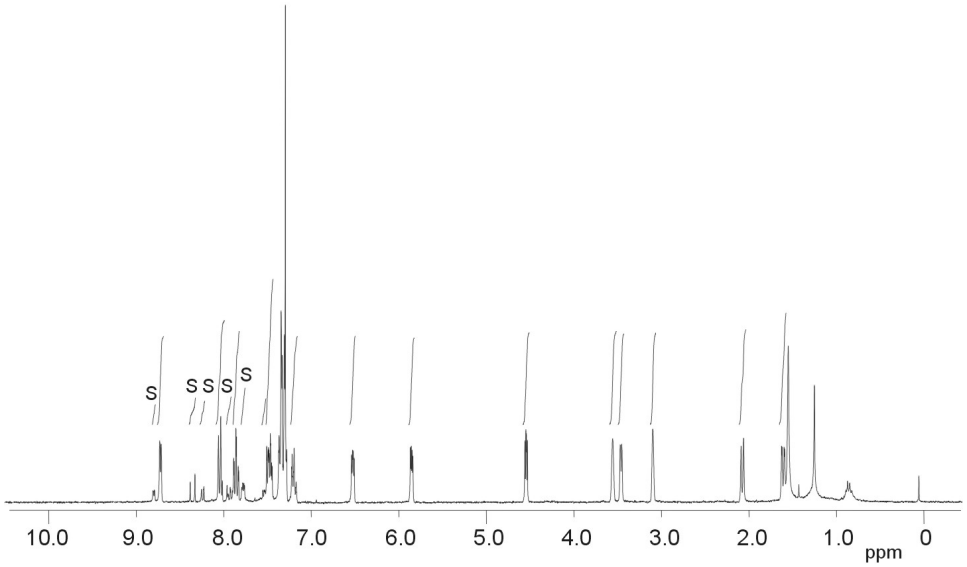

Figure S3. ^{1}H-NMR of the crude product **3a** of the Diels-Alder reaction catalyzed by [Cu(dmbipy)(NO_3)$_2$] (table 1, entry 16). S denotes residual starting material **2a**.

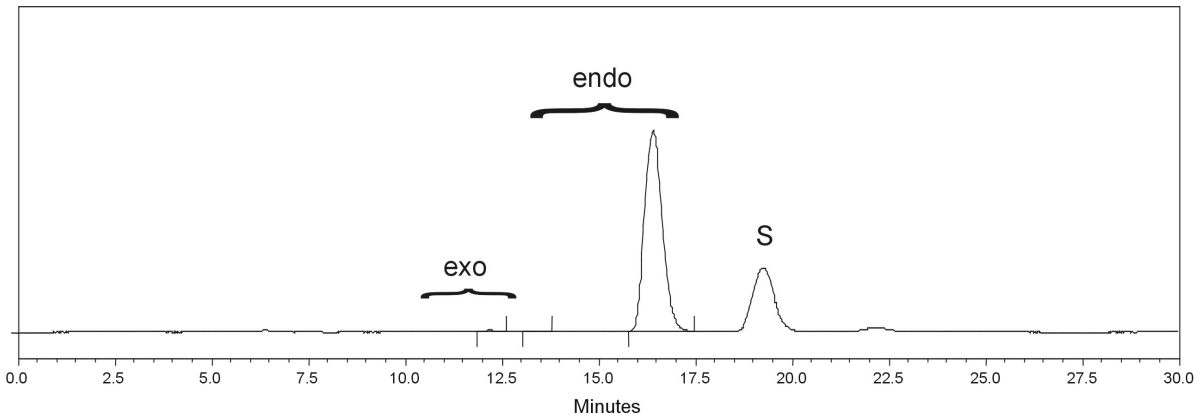

Figure S4. HPLC trace of the crude product **3a** of the Diels-Alder reaction catalyzed by [Cu(dmbipy)(NO_3)$_2$] (table 1, entry 16). S denotes residual starting material **2a**. The peak of one of the enantiomers of the exo product (at 11.4 min) is too small to detect.

Synthesis

General remarks
Dienophiles **2a-b**,[3] Cu(dppz)(NO_3)$_2$,[4] Cu(dpq)(NO_3)$_2$,[4] 2-(2-pyridyl)imidazole (**9**)[5] were prepared following published procedures.

(*E*)-4,4-dimethyl-1-(2-pyridinyl)-2-penten-1-one (2c). This compound was prepared following the procedure as described for **2a**.[3] Starting from 2-acetylpyridine (2.06 g, 17 mmol) and pivaldehyde (1.42 g, 16.5 mmol), after column chromatography (SiO_2, heptane/ethyl acetate 8:1), **2c** was obtained as a white solid. Yield: 706 mg, 3.7 mmol, 22 %). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.18 (s, 9H), 7.24 (dd, 1H, J = 15.8 Hz, J = 0.8 Hz), 7.46 (m, 1H), 7.54 (d, 1H, J = 16.5 Hz), 7.84 (m, 1H), 8.12 (d, 1H, J = 7.3 Hz), 8.71 (m, 1H). ^{1}H-NMR ($CDCl_3$, 100 MHz) δ 28.71 (q), 34.39 (s), 119.32 (d), 122.81 (d), 126.65 (d), 136.85 (d), 148.76 (d), 154.29 (s), 159.89 (d), 190.04 (d); Anal. Calcd for $C_{12}H_{15}NO$: C, 76.16 H, 7.99 N, 7.40. Found: C, 76.1 H, 8.04 N, 7.45.

[3-(*tert*-butyl)bicyclo[2.2.1]hept-5-en-2-yl](2-pyridinyl)methanone (**3c**, major isomer)
^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.89 (s, 9H), 1.38 (m, 1H), 1,80 (m, 1H), 1.90 (dd, 1H, J = 6.2 Hz, J = 1.5 Hz), 2.76 (dd, 1H, J = 2.9 Hz, J = 1.5 Hz), 3.22 (m, 1H), 4.26 (dd, 1H, J = 6.2 Hz, J = 2.9 Hz), 5.69 (dd, 1H, J = 5.5 Hz, J = 2.6 Hz), 6.43 (dd, 1H, J = 5.5 Hz, J = 2.9 Hz), 7.44 (m, 1H), 7.78 (m, 1H), 7.97 (d, 1H, J = 7.7 Hz), 8.71 Hz (d, 1H, J = 4.8 Hz); MS (CI): 256 (M+1); HRMS Calcd for $C_{17}H_{21}N_1O_1$ 255.1623, found 255.1613.

[Cu(phen)(NO_3)$_2$]. Following the procedure as described for [Cu(dppz)(NO_3)$_2$],[4] starting from phenanthroline (70 mg, 0.35 mmol) and Cu(NO_3)$_2$·3H_2O (94 mg, 1.1 eq), [Cu(phen)(NO_3)$_2$] was obtained as a blue solid. Yield: 114 mg, 0.31 mmol, 89 %. Anal. Calcd for $C_{12}H_8CuN_4O_6$: C, 39.19 H, 2.19 N, 15.23. Found: C, 39.25 H, 2.09 N, 15.15.

[Cu(bipy)(NO_3)$_2$]. Following the procedure as described for [Cu(dppz)(NO_3)$_2$], starting from 2,2'-bipyridine (60 mg, 0.39 mmol) and Cu(NO_3)$_2$·3H_2O (100 mg, 1.1 eq),[4] [Cu(bipy)(NO_3)$_2$] was obtained as a blue solid. Yield: 86 mg, 0.25 mmol, 64 %. Anal. Calcd for $C_{10}H_8CuN_4O_6$: C, 34.94 H, 2.35 N, 16.30. Found: C, 35.1 H, 2.30 N, 16.15.

[Cu(2-(2-pyridyl)imidazole)(NO_3)$_2$·H_2O]. To a solution of 2-(2-pyridyl)imidazole (**9**) (74 mg, 0.51 mmol) in ethanol (10 mL) was added Cu(NO_3)$_2$·3H_2O (123 mg, 0.51 mmol). The mixture was shaken until a clear green solution was obtained. The solution was placed in an ether bath for 2 days. The green crystals were filtered and washed with water and ethanol. Yield: 65 mg, 38%. Anal. Calcd for $C_8H_9CuN_5O_7$: C, 27.40 H, 2.59 N, 19.97. Found: C, 27.6 H, 2.49 N, 19.74.

[Cu(2-(2-pyridyl)benzimidazole)(NO_3)$_2$· H_2O]. To a solution of $Cu(NO_3)_2 \cdot 3H_2O$ (97 mg, 0.39 mmol) in a mixture of acetone (4 mL) and ethanol (0.3 ml) was added a solution of 2-(2-pyridyl)benzimidazole (**10**) (75 mg, 0.38 mmol) in ethyl acetate (4ml), through a small cotton plug. The dark green solution was filtered and the vial was closed with a cotton plug, allowing for slow evaporation of acetone. After one night a dark green solid had precipitated, which was washed with a small volume of ethyl acetate. Yield: 135 mg, 89 %. Anal. Calcd for $C_{12}H_{11}CuN_5O_7$: C, 35.96 H, 2.77 N, 17.47. Found: C, 36.30 H, 2.83 N, 17.04.

[Cu(4,4'-dimethyl-2,2'-dipyridyl)(NO_3)$_2$]. To a solution of $Cu(NO_3)_2 \cdot 3H_2O$ (0.10 g, 0.41 mmol) in ethanol was added 4,4'-dimethyl-2,2'-dipyridyl (**11**) (38 mg, 0.24 mmol), dissolved in ethanol. The solution was placed in an ethyl acetate bath and left standing for 2 days. The blue solid was filtered and washed with ethanol. Yield: 49 mg, 59%. Anal. Cald for $C_{10}H_8CuN_4O_6$: C, 38.8 H, 3.25 N, 15.07. Found: C, 38.5 H, 3.14 N, 14.80.

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